REPORT

DETERMINATION OF 'READY' BIODEGRADABILITY:

CARBON DIOXIDE (CO_2) EVOLUTION TEST (MODIFIED STURM TEST)

WITH

[redacted]

NOTOX Project 338759
NOTOX Substance 111834/B

STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with the most recent edition of:

The OECD Principles of Good Laboratory Practice

which are essentially in conformity with:

The United States Food and Drug Administration. Title 21 Code of Federal Regulations Part 58.

The United States Environmental Protection Agency (FIFRA). Title 40 Code of Federal Regulations Part 160.

The United States Environmental Protection Agency (TSCA). Title 40 Code of Federal Regulations Part 792.

Study Director

In [redacted]

.....

Date: June 26, 2002.

Management

[redacted]

Head of Genetic & Ecotoxicology

[redacted] Technical Director

.....

Date: 26/06/2002

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by [redacted]. Reproduction, issue or disclosure to third parties in any form is not permitted without prior written authorisation from the sponsor.

QUALITY ASSURANCE STATEMENT

NOTOX B.V., 's-Hertogenbosch, The Netherlands

This report was audited by the NOTOX Quality Assurance Unit to ensure that the methods and results accurately reflect the raw data.

The dates of Quality Assurance inspections and audits are given below.
During the on-site inspections procedures applicable to this type of study were inspected.

DATES OF QAU INSPECTIONS AUDITS	REPORTING DATES
on-site inspection (s)	
21 – 31 January 2002 (Process, Ecotoxicology & Biodegradation)	05 February 2002
protocol inspection (s)	
07 January 2002 (study)	07 January 2002
report audit (s)	
18 May 2002 (study)	18 May 2002

Head of Quality Assurance [redacted]ell B.Sc.

pp

J.T.G Witschi

Date: June 25, 2002

SUMMARY

[redacted] was tested for its ready biodegradability in the carbon dioxide (CO_2) evolution test (modified Sturm test) at approximately 44 mg per 2 litres, corresponding to 12 mg TOC/l.

The study procedure was based on EEC directive 92/69, C.4-C, December 1992, and OECD guideline No. 301 B July 17, 1992.

The Theoretical CO_2 production ($ThCO_2$) of TRIGONOX R-938 was calculated to be 2.01 mg CO_2/mg.

For this project two experiments were performed.

Since [redacted] was poorly soluble in water, weighed amounts of TRIGONOX R-938 were added to test bottles.
The weighed amounts of [redacted] added to the test bottles in experiment 1 were: 44.1 mg to test substance bottle A, 43.8 mg to test substance bottle B and 44.4 mg to the toxicity control bottle.
The weighed amounts of [redacted] added to the test bottles in experiment 2 were: 44.5 mg to test substance bottle A, 44.9 mg to test substance bottle B and 44.1 mg to the toxicity control bottle.
10 ml of milli-RO water was added to each weighing bottle and after vigorous shaking the resulting suspension was added quantitatively to the test medium.
The test solutions were continuously stirred during the test.

Experiment 1:
The relative degradation values calculated from the measurements performed during the test period revealed 44% degradation of [redacted] in test bottle A and 81% in test bottle B.

Experiment 2:
The relative degradation values calculated from the measurements performed during the test period revealed 91% degradation of [redacted] in test bottle A and 62% in test bottle B.

In both experiments, biodegradation of [redacted] of at least 60% was not reached within 10 days of biodegradation exceeding 10%. Thus, the criterion for ready biodegradability was not met.
In the toxicity control of both experiments [redacted] was found to be not inhibitory on microbial activity.

Except for the difference between the duplicate degradation values, all criteria for acceptability of the tests were met.
Since the difference of duplicate degradation values was observed in both experiments and all other acceptability criteria were met, the difference was considered to be test substance related. Furthermore, the criterion for ready biodegradability (at least 60% degradation within 10 days of biodegradation exceeding 10%) was not met. Thus, the difference of duplicate values has no influence on the final conclusion (the criterion for ready biodegradability).
Therefore, the studies were considered to be valid.

In conclusion, under the conditions of these tests [redacted] was not readily biodegradable.

EEC CRITERIA FOR CLASSIFICATION AND LABELLING

Based on the results and according to the EEC criteria for classification and labelling of dangerous substances and preparations (Commission Directive 93/21/EEC of 27 April 1993) [redacted] environment) if the log Pow is ≥ 3.0.

PREFACE

Sponsor	[redacted]
Study Monitor	[redacted] y Affairs
Testing Facility	NOTOX B.V. Hambakenwetering 7 5231 DD 's-Hertogenbosch The Netherlands
Study Director	Ing. M.J.E. Desmares-Koopmans
Technical Coordinator	J.H.J.W. Kluytmans
Study Plan	Start : January 09, 2002 Completion : March 14, 2002

TEST SUBSTANCE

Identification	[redacted]
Chemical name	[redacted] d
[redacted]	Clear colourless liquid
Batch	1510-14
Purity	See Certificate of Analysis
Test substance storage	In refrigerator in the dark
Stability under storage conditions	Stable
Expiry date	01 January 2003
Density	Approx. 1160 $kg.m^{-3}$
Stability in water	Unknown

The sponsor is responsible for all test substance data unless determined by NOTOX.

Note: **Don't heat up the test substance above 50°C**

Certificate of analysis is appended to the report.

PURPOSE

The purpose of the study is to evaluate a non-volatile test substance for its ready biodegradability in an aerobic aqueous medium with microbial activity introduced by inoculation of the supernatant of activated sludge.

GUIDELINES

The study procedure described in this report was based on the following guidelines:

European Economic Community (EEC), EEC directive 92/69, Part C: Methods for the determination of ecotoxicity, Publication No. L383, December 1992,
C.4. Biodegradation: determination of the 'ready' biodegradability,
C.4-C: Carbon dioxide (CO_2) evolution test (modified Sturm test).

Organisation for Economic Co-operation and Development (OECD), OECD guidelines for Testing of Chemicals, Section 3, Degradation and Accumulation, guideline No. 301 B: "Ready Biodegradability: CO_2 Evolution Test" adopted July 17, 1992.

ARCHIVING

NOTOX B.V. will archive the following data for at least 10 years: protocol, report, test substance reference sample and raw data.
No data will be withdrawn without the sponsor's written consent.

DEFINITIONS

Readily biodegradable	: Test substances giving a result of at least 60% yield of CO_2 (within 28 days). This pass level must be reached within 10 days of biodegradation exceeding 10%.
$ThCO_2$	: Theoretical carbon dioxide (mg) is the quantity of carbon dioxide calculated to be produced from the known or measured carbon content of the test substance when fully mineralized; also expressed as mg carbon dioxide evolved per mg test substance.
TOC	: Total organic carbon of a sample is the sum of the organic carbon in solution and in suspension.

INTRODUCTION

For this project two experiments were performed.
Both experiments will be discussed in this report.

PREPARATION OF TEST SOLUTIONS

Since [redacted] was poorly soluble in water, weighed amounts of [redacted] were added to test bottles.

The weighed amounts of [redacted] added to the test bottles in experiment 1 were: 44.1 mg to test substance bottle A, 43.8 mg to test substance bottle B and 44.4 mg to the toxicity control bottle.
The weighed amounts of [redacted] added to the test bottles in experiment 2 were: 44.5 mg to test substance bottle A, 44.9 mg to test substance bottle B and 44.1 mg to the toxicity control bottle.

10 ml of milli-RO water was added to each weighing bottle and after vigorous shaking the resulting suspension was added quantitatively to the test medium.
The test solutions were continuously stirred during the test.

TEST SYSTEM

Source	The source of test organisms was activated sludge freshly obtained from a municipal sewage treatment plant: 'Waterschap de Maaskant', 's-Hertogenbosch, the Netherlands.
Treatment	The sludge was kept under continuous aeration until further treatment. The concentration of suspended solids was 3.8 g/l in the concentrated sludge in experiment 1 and 3.7 g/l in the concentrated sludge in experiment 2 (information obtained from the municipal sewage treatment plant). Before use, the sludge was allowed to settle (30-90 minutes) and the liquid decanted for use as inoculum at the amount of 10 ml/l of mineral medium.
Reason for selection	The test has been accepted internationally (EEC, OECD) for determining the 'ready' biodegradability of test substances under aerobic conditions.

TEST PROCEDURE AND CONDITIONS

Test duration	28 days (last CO_2-measurement on the 29th day). During the test period aeration and stirring took place.
Test vessels	2 litre all-glass brown coloured bottles.
Milli-RO / Milli-Q water	Tap-water purified by reverse osmosis (Milli-RO) and subsequently passed over activated carbon and ion-exchange cartridges (Milli-Q) (Millipore Corp., Bedford, Mass., USA).
Stock solutions of mineral components	A) 8.50 g KH_2PO_4 21.75 g K_2HPO_4 67.20 g $Na_2HPO_4.12H_2O$ 0.50 g NH_4Cl dissolved in 1 l Milli-Q water, pH 7.4 $\pm$ 0.2 B) 22.50 g $MgSO_4.7H_2O$ dissolved in 1 l Milli-Q water. C) 36.40 g $CaCl_2.2H_2O$ dissolved in 1 l Milli-Q water. D) 0.25 g $FeCl_3.6H_2O$ dissolved in 1 l Milli-Q water.
Mineral medium	1 l mineral medium contains: 10 ml of solution (A), 1 ml of solutions (B) to (D) and Milli-RO water.
Barium hydroxide	0.0125 M, stored in a sealed vessel to prevent absorption of CO_2 from the air.

CO_2-free air	A mixture of oxygen (21%) and nitrogen (79%) was led through a bottle, containing 0.5 - 1 litre 0.0125 M $Ba(OH)_2$ solution to trap CO_2 which might be present in small amounts. The CO_2-free air was sparged through the scrubbing solutions at a rate of approximately 1-2 bubbles per second (ca. 30-100 ml/min).
Test concentration	The test substance was tested in duplicate at approximately 44 mg per 2 litres, corresponding to 12 mg TOC/l. The organic carbon content was based on the composition of the test substance and the molecular formulae of the components.
Preparation of bottles:	
Pre-incubation medium	Mineral components, Milli-RO water (ca. 80% total volume) and inoculum (1% final volume) were added to each bottle. This mixture was aerated with CO_2-free air overnight to purge the system of CO_2.
Type and number of bottles	Test suspension: containing test substance and inoculum (2 bottles). Inoculum blank: containing only inoculum (2 bottles) Positive control: containing reference substance (ca. 40 mg/l sodium acetate (Merck art. 1062680250, batch TA 820068 033), TOC= 12 mg/l) and inoculum (1 bottle). Toxicity control: containing test substance, reference substance and inoculum (1 bottle).
Preparation	The test substance and positive control were added to the bottles. The volumes of suspensions were made up to 2 litres with Milli-RO water, resulting in the mineral medium described before. Three CO_2-absorbers (bottles filled with 100 ml 0.0125 M $Ba(OH)_2$) were connected in series to the exit air line of each test bottle.
Start of the incubation	The test was started by bubbling CO_2-free air through the solution at a rate of approximately 1-2 bubbles per second (ca. 30-100 ml/min).

DETERMINATION OF CO_2

Experimental CO_2 production	The CO_2 produced in each test bottle reacted with the barium hydroxide in the gas scrubbing bottle and precipitated out as barium carbonate. The amount of CO_2 produced was determined by titrating the remaining $Ba(OH)_2$ with 0.05 M standardized HCl.
Measurements	Titrations were made every second or third day during the first 10 days, and thereafter at least every fifth day until the 28th day[1]. Each time the CO_2-absorber nearest to the test bottle was removed for titration; each of the remaining two absorbers was moved one position in the direction of the test bottle. A new CO_2-absorber was placed at the far end of the series. Phenolphthalein was used as pH-indicator. On the 28th day, the pH of the test suspensions was measured and 1 ml of concentrated HCl was added to each bottle. The bottles were aerated overnight to drive off CO_2 present in the test suspension. The final titration was made on day 29.
Theoretical CO_2 production	The theoretical CO_2 production was calculated from the composition of the test substance and the molecular formulae of the components.

[1] Inadvertently no titration was made on nominal day 19 of the first experiment. However, this missing titration was considered to have no effect on the outcome of this experiment.

DATA EVALUATION

$ThCO_2$, expressed as mg CO_2/mg test substance, that can be generated by a test substance was calculated as follows:

$$ThCO_2 = \frac{\text{No. of carbon atoms in test substance x MW } CO_2}{\text{MW test substance}}$$

After calculation of the $ThCO_2$ for each component the total $ThCO_2$ was calculated.

The first step in calculating the amount of CO_2 produced is to correct for background (endogeneous) CO_2 production. Thus the amount of CO_2 produced by a test material is determined by the difference (in ml of titrant) between the experimental and blank $Ba(OH)_2$ traps.

The amount of 0.05 N HCl titrated is converted into mg of CO_2 produced:

$$\text{mg } CO_2 = \frac{(0.05 \text{ x ml HCl titrated})}{2} \text{ x } 44 = 1.1 \text{ x ml of HCl titrated.}$$

Calculations were based on the actual normality without rounding off.
Relative degradation values were calculated from the cumulative CO_2 production relative to the total expected CO_2 production based on the total carbon content of the amount of test material present in the test bottles. They were plotted versus time together with the relative degradation of the positive control.

A figure of more than 10% degradation was considered as significant.

Toxicity control: if less than 25% degradation (based on $ThCO_2$) occurred within 14 days, the test substance can be assumed to be inhibitory.

ACCEPTABILITY OF THE TEST

The results of the biodegradation test were considered to be valid when:

- the total CO_2 evolution in the inoculum blank at the end of the test did not normally exceed 40 mg/l. If values greater than 70 mg CO_2/l are obtained, the data and experimental technique should be examined critically.
  For the calculation of the total CO_2 evolution in the inoculum blank the volume of HCl needed for the titrating of the remaining $Ba(OH)_2$ of the inoculum blanks and for fresh $Ba(OH)_2$ was used.

- the difference of duplicate values for the %-degradation of the test substance at the plateau, at the end of the test or at the end of the 10-day window, as appropriate, was less than 20.

- the percentage degradation of the reference substance reached the level for ready biodegradability (60%) by 14 days.

Because of the stringency of the method, low values do not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates more work will be necessary to establish biodegradability.

RESULTS

Theoretical CO_2 production

The Theoretical CO_2 production ($ThCO_2$) of [redacted] was calculated to be 2.01 mg CO_2/mg, based on the information given in the schedule below and the appended certificate of analysis.

	%C:	$ThCO_2$:	Average %:
[redacted]	44.4	1.63	18.6
	50.8	1.86	7.9
	0	0	2.1
	61.8	2.27	67.0
	68.1	2.50	2.0
	0	0	2.6
impurities	unknown	unknown	0.5 neglectible

Experiment 1:

- The concentration was 44.1 (A) and 43.8 mg (B) [redacted] in 2 litres test medium. Hence, the theoretical CO_2 production following complete degradation was 88.6 mg per 2 litres for A and 88.0 mg per 2 litres for B.
- The positive control contained 79.1 mg sodium acetate ($ThCO_2$= 1.07 mg CO_2/mg) resulting in a theoretical CO_2 production following complete degradation of 84.6 mg per 2 litres.
- The toxicity control contained 79.1 mg sodium acetate and 44.4 mg [redacted] complete degradation of [redacted] 8 plus sodium acetate was 173.9 mg per 2 litres.

Experiment 2:

- The concentration was 44.5 (A) and 44.9 mg (B) [redacted] in 2 litres test medium. Hence, the theoretical CO_2 production following complete degradation was 89.4 mg per 2 litres for A and 90.2 mg per 2 litres for B.
- The positive control contained 80.8 mg sodium acetate ($ThCO_2$= 1.07 mg CO_2/mg) resulting in a theoretical CO_2 production following complete degradation of 86.5 mg per 2 litres.
- The toxicity control contained 80.8 mg sodium acetate and 44.1 mg TRIGONOX R-938 in 2 litres of test medium. Hence, the theoretical CO_2 production following complete degradation of [redacted] plus sodium acetate was 175.1 mg per 2 litres.

Biodegradation

The results of experiment 1 are summarized in Tables 1-4, and Figure 1.
The results of experiment 2 are summarized in Tables 5-8, and Figure 2.

Experiment 1:
The relative degradation values calculated from the measurements performed during the test period revealed 44% degradation of [redacted] in test bottle A and 81% in test bottle B. However, biodegradation of [redacted] of at least 60% was not reached within 10 days of biodegradation exceeding 10%. Thus, the criterion for ready biodegradability was not met.

Since the difference of the relative degradation values for A and B was not always less than 20 a second experiment was performed.

Experiment 2:
The relative degradation values calculated from the measurements performed during the test period revealed 91% degradation of [redacted] in test bottle A and 62% in test bottle B. However, biodegradation of [redacted] of at least 60% was not reached within 10 days of biodegradation exceeding 10%. Thus, the criterion for ready biodegradability was not met.

In the second experiment a difference of duplicate values for %-degradation of [redacted] of 20% or more was noted again.

In the toxicity control of both experiments more than 25% degradation occurred within 14 days (based on $ThCO_2$). Therefore, the test substance was assumed to be not inhibitory on microbial activity.

Monitoring of temperature and pH

The temperature recorded in a vessel with water in the same room varied between 22 and 23°C in both experiments.

Experiment 1:
The pH values of the different test media were:

		Just before the start of the test:	On day 28:
Blank control (A)	:	7.6	7.7
Blank control (B)	:	7.6	7.7
Positive control	:	7.6	8.3
[redacted]	:	7.6	7.7
Toxicity control	:	7.6	8.0

Experiment 2:
The pH values of the different test media were:

		Just before the start of the test:	On day 28:
Blank control (A)	:	7.5	7.5
Blank control (B)	:	7.5	7.5
Positive control	:	7.4	7.8
[redacted]	:	7.4	7.5
Toxicity control	:	7.4	7.7

Acceptability of the test

Experiment 1:
- The positive control substance was degraded 60% within 9 days (see Table 1 and Figure 1).
- The total CO_2 release in the blank reached a total value of 51 mg CO_2 per 2 litres of medium (see Table 4).
- The difference of duplicate values for %-degradation of [redacted] was not always less than 20 (Δ A-B $\geq$ 20% on days 9, 23, 26, 27 and 29, see Table 2c).

Except for the difference of duplicate values for %-degradation of [redacted], all criteria for acceptability of the test were met.

Experiment 2:
- The positive control substance was degraded at least 60% within 7 days (see Table 5 and Figure 2).
- The total CO_2 release in the blank reached a total value of 28 mg CO_2 per 2 litres of medium (see Table 8).
- Except on days 14 and 29, the difference of duplicate values for %-degradation of [redacted] was always less than 20 (see Table 6c).

Except for the difference of duplicate values for %-degradation of [redacted] all criteria for acceptability of the test were met.

DISCUSSION

As described previously, in both experiments a difference of the relative degradation values for A and B of more than 20% was noted. Therefore, one acceptability criterion as prescribed by the protocol was not met.

Since the difference of duplicate degradation values was observed in both experiments and all other acceptability criteria were met, the difference was considered to be test substance related.

Furthermore, the criterion for ready biodegradability (at least 60% degradation within 10 days of biodegradation exceeding 10%) was not met. Thus, the difference of duplicate values has no influence on the final conclusion (the criterion for ready biodegradability).
Therefore, the studies were considered to be valid.

CONCLUSION

[redacted]8 was degraded significantly (44, 62, 81 and 91%) during the test period. However, since at least 60% biodegradation was not reached within ten days of biodegradation exceeding 10%, the criterion for ready biodegradability was not met. Thus, under the conditions of this test [redacted] was not readily biodegradable.

EXPERIMENT 1:

Note: All calculations were performed without rounding off.

Table 1: Experiment 1 - CO_2 production and percentage biodegradation of the positive control substance.

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2	Degradation [1)]
	Blank (mean)	Positive control	(ml HCl)	(mg)	(mg)	(%)
0	-	-	-	-	-	0
2	42.82	27.18	15.64	17.2	17.2	20
5	42.69	25.53	17.16	18.9	36.1	43
7	43.21	34.85	8.36	9.2	45.3	53
9	43.93	38.65	5.28	5.8	51.1	60
14	43.79	36.56	7.23	8.0	59.0	70
23	44.62	35.77	8.84	9.7	68.8	81
26	43.89	42.98	0.91	1.0	69.7	82
27	44.82	46.52	0.00	0.0	69.7	82
29	45.90	42.64	3.26	3.6	73.3	87
29	48.10	48.23	0.00	0.0	73.3	87
29	47.64	49.22	0.00	0.0	73.3	87

1): Calculated as the ratio between CO_2 produced (cumulative) and the $ThCO_2$ of sodium acetate: 84.6 mg CO_2/2l

Table 2a: Experiment 1 - CO_2 production and percentage biodegradation of the test substance (bottle A).

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2	Degradation [1)]
	Blank (mean)	bottle A	(ml HCl)	(mg)	(mg)	(%)
0	-	-	-	-	-	0
2	42.82	43.13	0.00	0.0	0.0	0
5	42.69	43.52	0.00	0.0	0.0	0
7	43.21	32.60	10.61	11.7	11.7	13
9	43.93	36.95	6.98	7.7	19.3	22
14	43.79	30.94	12.85	14.1	33.5	38
23	44.62	39.27	5.34	5.9	39.4	44
26	43.89	45.73	0.00	0.0	39.4	44
27	44.82	47.60	0.00	0.0	39.4	44
29	45.90	46.07	0.00	0.0	39.4	44
29	48.10	48.39	0.00	0.0	39.4	44
29	47.64	49.51	0.00	0.0	39.4	44

1): Calculated as the ratio between CO_2 produced (cumulative) and the $ThCO_2$ of the test substance: 88.6 mg CO_2/2l

Table 2b: Experiment 1 - CO_2 production and percentage biodegradation of the test substance (bottle B).

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2	Degradation [1)]
	Blank (mean)	bottle B	(ml HCl)	(mg)	(mg)	(%)
0	-	-	-	-	-	0
2	42.82	43.54	0.00	0.0	0.0	0
5	42.69	40.72	1.97	2.2	2.2	2
7	43.21	24.64	18.57	20.4	22.6	26
9	43.93	29.86	14.07	15.5	38.1	43
14	43.79	34.15	9.64	10.6	48.7	55
23	44.62	33.40	11.22	12.3	61.0	69
26	43.89	38.24	5.65	6.2	67.2	76
27	44.82	42.45	2.36	2.6	69.8	79
29	45.90	44.27	1.63	1.8	71.6	81
29	48.10	48.26	0.00	0.0	71.6	81
29	47.64	48.85	0.00	0.0	71.6	81

1): Calculated as the ratio between CO_2 produced (cumulative) and the $ThCO_2$ of the test substance: 88.0 mg CO_2/2l

Table 2c: Experiment 1 - Comparison of biodegradation of the test substance in bottles A and B

Day	Biodegradation (%)			
	Bottle A	Bottle B	Mean A and B	Δ A-B [1)]
0	0	0	0	0
2	0	0	0	0
5	0	2	1	2
7	13	26	19	12
9	22	43	33	21
14	38	55	47	18
23	44	69	57	25
26	44	76	60	32
27	44	79	62	35
29	44	81	63	37
29	44	81	63	37
29	44	81	63	37

1): Absolute difference in biodegradation between bottles A and B

Table 3: Experiment 1 - CO_2 production and percentage biodegradation of the toxicity control.

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2	Degradation [1)]
	Blank (mean)	toxicity control	(ml HCl)	(mg)	(mg)	(%)
0	-	-	-	-	-	0
2	42.82	35.49	7.33	8.1	8.1	5
5	42.69	34.55	8.14	8.9	17.0	10
7	43.21	39.14	4.07	4.5	21.5	12
9	43.93	26.55	17.38	19.1	40.6	23
14	43.79	20.46	23.33	25.7	66.3	38
23	44.62	18.62	26.00	28.6	94.9	55
26	43.89	38.87	5.02	5.5	100.4	58
27	44.82	45.16	0.00	0.0	100.4	58
29	45.90	28.92	16.98	18.7	119.0	68
29	48.10	47.81	0.28	0.3	119.4	69
29	47.64	49.69	0.00	0.0	119.4	69

1): Calculated as the ratio between CO_2 produced (cumulative) and the sum of the $ThCO_2$ of the test substance and positive control:

$ThCO_2$ test substance: 89.2 mg CO_2/2l

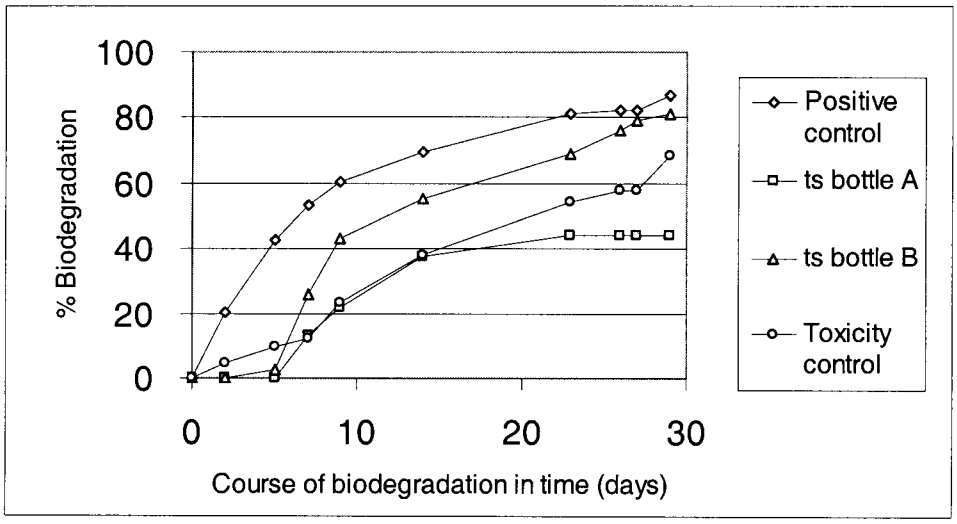
$ThCO_2$ sodium acetate: 84.6 mg CO_2/2l

Table 4: Experiment 1 - CO_2 production in the blank.

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2
	$Ba(OH)_2$ [1)]	Blank (mean)	(ml HCl)	(mg)	(mg)
0	-	-	-	-	0.0
2	48.28	42.82	5.47	6.0	6.0
5	48.63	42.69	5.94	6.5	12.6
7	47.87	43.21	4.67	5.1	17.7
9	48.13	43.93	4.20	4.6	22.3
14	47.27	43.79	3.48	3.8	26.1
23	49.61	44.62	5.00	5.5	31.6
26	49.94	43.89	6.05	6.7	38.3
27	50.15	44.82	5.34	5.9	44.1
29	49.42	45.90	3.52	3.9	48.0
29	49.12	48.10	1.03	1.1	49.1
29	49.41	47.64	1.77	1.9	51.1

1): "Strength" of untreated 0.0125 M $Ba(OH)_2$ solution

Figure 1: Experiment 1 - Biodegradation of [redacted] in the modified Sturm test.



EXPERIMENT 2:

Note: All calculations were performed without rounding off.

Table 5: Experiment 2 - CO_2 production and percentage biodegradation of the positive control substance.

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2	Degradation [1)]
	Blank (mean)	Positive control	(ml HCl)	(mg)	(mg)	(%)
0	-	-	-	-	-	0
2	46.06	37.03	9.03	9.9	9.9	11
5	46.55	19.29	27.26	30.0	39.9	46
7	46.53	32.25	14.28	15.7	55.6	64
9	48.12	39.47	8.65	9.5	65.1	75
14	47.89	40.13	7.76	8.5	73.7	85
19	46.73	41.67	5.06	5.6	79.2	92
23	45.09	44.16	0.93	1.0	80.2	93
27	46.85	45.71	1.14	1.2	81.5	94
29	46.16	44.98	1.18	1.3	82.8	96
29	47.71	47.68	0.03	0.0	82.8	96
29	48.32	48.71	0.00	0.0	82.8	96

[1)]: Calculated as the ratio between CO_2 produced (cumulative) and the $ThCO_2$ of sodium acetate: 86.5 mg CO_2/2l

Table 6a: Experiment 2 - CO_2 production and percentage biodegradation of the test substance (bottle A).

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2	Degradation [1)]
	Blank (mean)	bottle A	(ml HCl)	(mg)	(mg)	(%)
0	-	-	-	-	-	0
2	46.06	43.79	2.27	2.5	2.5	3
5	46.55	42.76	3.79	4.2	6.7	7
7	46.53	44.23	2.30	2.5	9.2	10
9	48.12	45.92	2.20	2.4	11.6	13
14	47.89	47.10	0.79	0.9	12.5	14
19	46.73	24.19	22.54	24.8	37.3	42
23	45.09	25.78	19.31	21.2	58.5	65
27	46.85	37.82	9.03	9.9	68.4	77
29	46.16	40.35	5.81	6.4	74.8	84
29	47.71	42.74	4.97	5.5	80.3	90
29	48.32	47.70	0.62	0.7	81.0	91

[1)]: Calculated as the ratio between CO_2 produced (cumulative) and the $ThCO_2$ of the test substance: 89.4 mg CO_2/2l

Table 6b: Experiment 2 - CO_2 production and percentage biodegradation of the test substance (bottle B).

Day	HCl (0.05 N) titrated (ml)		Produced CO_2	Produced CO_2	Cumulative CO_2	Degradation [1)]
	Blank (mean)	bottle B	(ml HCl)	(mg)	(mg)	(%)
0	-	-	-	-	-	0
2	46.06	43.28	2.78	3.1	3.1	3
5	46.55	43.17	3.38	3.7	6.8	7
7	46.53	45.54	0.99	1.1	7.9	9
9	48.12	46.22	1.90	2.1	9.9	11
14	47.89	29.09	18.80	20.7	30.6	34
19	46.73	33.02	13.71	15.1	45.7	51
23	45.09	37.67	7.42	8.2	53.9	60
27	46.85	45.14	1.71	1.9	55.7	62
29	46.16	46.18	0.00	0.0	55.7	62
29	47.71	48.75	0.00	0.0	55.7	62
29	48.32	48.28	0.04	0.0	55.8	62

[1)]: Calculated as the ratio between CO_2 produced (cumulative) and the $ThCO_2$ of the test substance: 90.2 mg CO_2/2l

Table 6c: Experiment 2 - Comparison of biodegradation of the test substance in bottles A and B

Day	Biodegradation (%)			
	Bottle A	Bottle B	Mean A and B	Δ A-B [1)]
0	0	0	0	0
2	3	3	3	1
5	7	7	7	0
7	10	9	9	2
9	13	11	12	2
14	14	34	24	20
19	42	51	46	9
23	65	60	63	6
27	77	62	69	15
29	84	62	73	22
29	90	62	76	28
29	91	62	76	29

1): Absolute difference in biodegradation between bottles A and B

Table 7: Experiment 2 - CO_2 production and percentage biodegradation of the toxicity control.

Day	HCl (0.05 N) titrated (ml)		Produced CO_2 (ml HCl)	Produced CO_2 (mg)	Cumulative CO_2 (mg)	Degradation [1)] (%)
	Blank (mean)	toxicity control				
0	-	-	-	-	-	0
2	46.06	46.76	0.00	0.0	0.0	0
5	46.55	26.86	19.69	21.7	21.7	12
7	46.53	32.22	14.31	15.7	37.4	21
9	48.12	39.98	8.14	8.9	46.3	26
14	47.89	16.50	31.39	34.5	80.9	46
19	46.73	25.80	20.93	23.0	103.9	59
23	45.09	40.13	4.96	5.5	109.3	62
27	46.85	44.44	2.41	2.6	112.0	64
29	46.16	37.82	8.34	9.2	121.2	69
29	47.71	47.34	0.37	0.4	121.6	69
29	48.32	48.43	0.00	0.0	121.6	69

1): Calculated as the ratio between CO_2 produced (cumulative) and the sum of the $ThCO_2$ of the test substance and positive control: 175.1 mg CO_2/2l

$ThCO_2$ test substance: 88.6 mg CO_2/2l

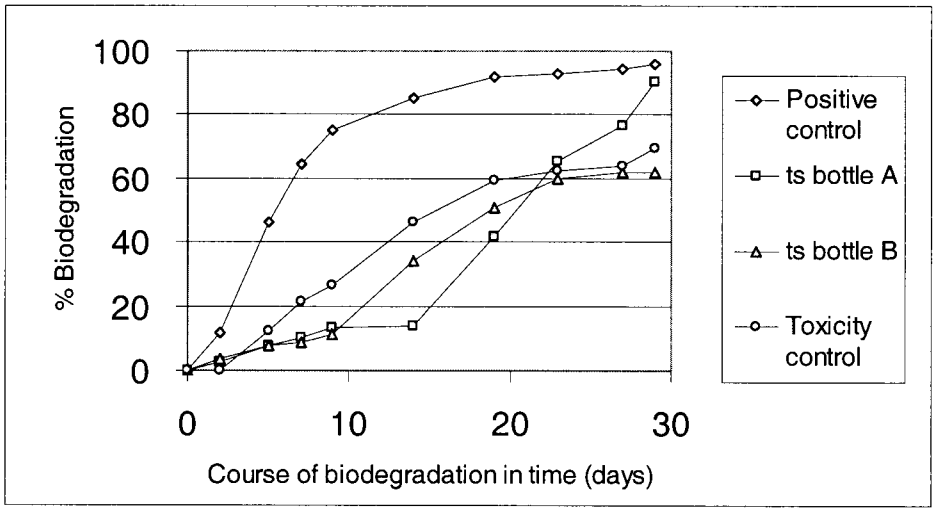
$ThCO_2$ sodium acetate: 86.5 mg CO_2/2l

Table 8: Experiment 2 - CO_2 production in the blank.

Day	HCl (0.05 N) titrated (ml)		Produced CO_2 (ml HCl)	Produced CO_2 (mg)	Cumulative CO_2 (mg)
	$Ba(OH)_2$ [1)]	Blank (mean)			
0	-	-	-	-	0.0
2	48.73	46.06	2.68	2.9	2.9
5	48.34	46.55	1.80	2.0	4.9
7	46.63	46.53	0.10	0.1	5.0
9	50.08	48.12	1.97	2.2	7.2
14	51.23	47.89	3.34	3.7	10.9
19	49.63	46.73	2.90	3.2	14.1
23	49.95	45.09	4.87	5.4	19.4
27	49.84	46.85	2.99	3.3	22.7
29	48.07	46.16	1.92	2.1	24.8
29	50.42	47.71	2.71	3.0	27.8
29	48.51	48.32	0.19	0.2	28.0

1): "Strength" of untreated 0.0125 M $Ba(OH)_2$ solution

Figure 2: Experiment 2 - Biodegradation of [redacted] in the modified Sturm test.



[redacted]

Certificate of Analysis [redacted]

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ICS-331

Product name : [redacted]

Chemical name : [redacted])

Batch number : **1510-14**

Test results:

Method	Analysis of	Unit	Result *1
Jo/72.11. [redacted]	[redacted] ***tication***		
J20010792	[redacted]	% m/m	67.0 (± 1.0)
[redacted]			
[redacted]			
[redacted]	s	% m/m	0.5 (± 0.2)

*1 bracketed values are estimated 95% confidence intervals

File code : **TNA-2001007**

Analytical documentation : 20010792

Authorized by

Name : D[redacted]
Function : Section Head, Analytical Research Department
Date : October 25, 2001

Signature :

[redacted]

[redacted]

Certificate of Analysis

[redacted]s

[redacted]

structure	% m/m
[redacted] (Type IV) IUPAC : [redacted]	18.6
[redacted] (Type III) IUPAC : [redacted]	7.9
[redacted]	2.1

[redacted]